Antibody Levels to Whole Culture Filtrate Antigens and to Purified P32 during Treatment of Smear-Positive Tuberculosis

Annie Drowart, M.D.; Kris Huygen, Ph.D.; Jacqueline De Bruyn, M.Sc.; Jean-Claude Yernault, M.D., Ph.D., F.C.C.P.; Claire-Michèle Farber, M.D., Ph.D.; and Jean-Paul Van Vooren, M.D., Ph.D.

Antimycobacterial IgG levels were measured repeatedly during treatment in 12 patients with moderate or severe pulmonary tuberculous disease using a dot immunobinding assay. We used reflectance densitometry equipment to quantify the immunoperoxidase staining and a Mycobacterium bovis BCG culture filtrate and the purified P32 protein as antigens. Antibody response against whole culture filtrate and P32 antigen increased after a three-month period of treatment. After this antibiotic therapy was completed, the estimated amount of antibodies directed against the P32 decreased while those against the whole culture filtrate remained at the same level. A serologic test using P32 as the antigen seems to allow a better discrimination between active and healed tuberculosis. (Chest 1991; 100:683-87)

In developed countries, the incidence of mycobacterial infections, low during the last years, is now slowly on the increase partly secondary to the emergence of the acquired immunodeficiency syndrome. On the other hand, human tuberculosis remains a major disease in developing countries and is frequently the direct cause of death. Laboratory diagnosis is difficult: microscopic examination of sputum samples may give less than 50 percent of positivity and culture techniques, more sensitive and providing a definitive diagnosis, require complex media. Standard roentgenograms are rarely performed during treatment. Thus, the only criteria to determine the efficacy of an antituberculosis treatment remain the clinical signs and disappearance of Mycobacteria on direct examination.

The development of the enzyme-linked immunosorbent assay (ELISA) and more recently the immunoblotting assay stimulated a new interest in studying humoral responses for the diagnosis of tuberculous infections. Many antigenic preparations and also purified antigens were screened for their potential interest in humoral tests. We used the immunoblotting techniques we had developed to perform such a study comparing the humoral responses directed against a whole highly immunoreactive culture filtrate and the P32 antigen.

Subjects and Methods

Control Group

The control sera were obtained from 40 healthy tuberculin skin test-negative subjects or 46 healthy tuberculin skin test-positive subjects after BCG vaccination or previous first infection.

Patients

Pulmonary tuberculosis was diagnosed in 12 patients by direct examination of sputum smear further confirmed by standard culture. All of them presented with moderate (eight patients) or severe (four patients) cavitory radiologic lesions. Serum samples were obtained at the time of diagnosis, during (±3 months later), and at the end (±9 months) of a conventional well-followed treatment (pyrazinamide, isoniazid, and rifampicin during the first three months, isoniazid and rifampicin during the following three months, and finally isoniazid alone during the last three months). Radiologic and clinical signs of disease activity disappeared in all patients after treatment was completed. The culture technique identified Mycobacterium tuberculosis sensitive to all used antimycobacterial agents in each case.

Antigens

Culture filtrate was obtained from a 15-day-old BCG 1173P2 M. bovis culture medium. P32 is a recently characterized highly immunoreactive mycobacterial antigen. It is present in large amounts in culture filtrate and corresponds to the 85A part of the 85 complex, previously described using crossed immunoelectrophoresis.

Dot Immunobinding Assay

Dot immunobinding assay (DIBA) was performed as previously described. This technique is known to give the same results as the commonly used ELISA technique. Specific antimycobacterial IgG antibody levels were evaluated. A densitometer (Bio Rad Laboratories) was used in reflectance conditions to quantify the peroxidase staining on the nitrocellulose support. Each sample was tested in triplicate. Three high and two low reactive serum samples were diluted serially and tested using each antigen. Reference curves were set up that were linear between 0.05 and 0.5 arbitrary determined reflectance units (RU). A 1:400 serum dilution was chosen to test all samples using culture filtrate as the antigen and a 1:200 dilution to test their reactivities against the purified P32 antigen. A cutoff point for positivity was determined corresponding to the 95th percentile of the values measured in the control group (n = 86).
**Statistics**

A Student's *t* test for paired values was performed to compare the antibody levels measured at each time of sampling.

**RESULTS**

**Whole Culture Filtrate Antigens (Fig 1)**

A cutoff point of 0.22 RU was determined using the 86 control sera. Nine patients showed a higher level of specific anticulture filtrate IgG antibodies at the beginning of the treatment. The levels rose after a three-month treatment period (*p*<0.001) so that ten of the patients showed "positive" values. These levels remained unchanged until the end of the treatment (no statistical difference between the values measured after three months and those measured after nine months).

**P32 Antigen (Fig 2)**

A 0.20 RU cutoff point was determined. Before starting the treatment eight patients had higher antibody levels than the control group. Those values slightly increased after the first three-month period (*p*<0.05), but decreased later so that the values of anti-P32 antibody levels were lower at the end of the treatment than those measured during the treatment (*p*<0.001) and at the time of diagnosis (*p*<0.05).
DISCUSSION

We report the evolution of specific antibody levels in the sera of 12 patients presenting with well-characterized cavitory pulmonary lesions at the time of diagnosis. All the patients were treated during nine months leading to a complete healing.

Whole mycobacterial culture filtrates or extracts have been widely used for the serodiagnosis of tuberculous diseases. Such preparations contain various antigens; some of them, P64 for example, are ubiquitous and more immunoreactive than mycobacterial-specific antigens such as P32. To improve the specificity of serologic tests, we and many other authors tried to purify the antigens or to produce them by cloning and expression of the corresponding genes. P32 corresponds to the 85A component of the 85 complex. A similar coding nucleotide sequence has been described recently in both M tuberculosis and M bovis BCG. The 85B antigenically related part of this complex called 30,000 Da antigen has been used in a serologic evaluation showing a 0.695 sensitivity in a comparable group of smear-positive patients.

The levels of antibodies directed against mycobacterial antigens were elevated at the beginning of treatment in nine (75 percent) of 12 patients using culture filtrate and in eight (66 percent) of 12 patients using P32 as antigen. A similar sensitivity was obtained in patients with moderate or severe disease by ELISA technique using the same P32 as antigen. Those levels rose after a few months of a well-conducted treatment. Several mechanisms causing such a phenomenon can be advocated such as intense stimulation of the humoral response by the antigens released from killed mycobacteria combined with a disinhibition of the immune system and/or the disappearance of circulating mycobacterial antigens so that specific antibodies are no longer trapped into immune complexes. We observed the same increase using both whole culture filtrate and P32 as antigen.

In developing countries, many physicians encounter the problem of making a correct diagnosis among the following: well-treated and healed tuberculosis in a patient whose clinical alteration is secondary to another unrelated disease; a failure in the antmycobacterial treatment; or an early relapse. To be of use, a serologic test should give information about the disease activity. Many authors, including ourselves, have reported large series of patients with newly diagnosed untreated tuberculosis whose sera contained higher levels of antmycobacterial antibodies than those of healthy subjects. There is limited information on the persistence of antibody after successful treatment. A study of Daniel et al performed on a limited number of eight patients using antigen 5 suggested that detectable antibody persisted for about two years after the onset of therapy.

We stress the importance of choosing an antigen like P32 inducing humoral response that evolves in parallel with disease activity to develop a useful serologic test for the diagnosis of tuberculosis.

REFERENCES

1 Chaissen RE, Slutkin G. Tuberculosis and human immunodeficiency virus infection. J Infect Dis 1989; 159:96-100
9 Sada E, Ferguson LE, Daniel TM. An ELISA for the serodiagnosis of tuberculosis using a 30000 Da native antigen of Mycobacterium tuberculosis. J Infect Dis 1990; 162:298-31
12 De Wit L, de la Cuvelerie A, Ooms J, Content J. Nucleotide sequence of the 32-kDa-protein gene (antigen 55A) of Mycobacterium bovis BCG. Nucleic Acid Res 1990; 18:3995